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Intracerebral transplantation: basic and clinical applications to the neostriatum

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ABSTRACT Many studies have used the intracerebral transplantation technique to study the neostriatum. Most of this work has been conducted in two well-characterized animal models of striatal dysfunction: the rat model of Huntington's disease (striatal damage) and the rat model of Parkinson's disease (damage of dopaminergic nigrostriatal afferents). In animals with striatal damage, fetal striatal tissue implanted into the neostriatum (homotypic transplants) displays a remarkable anatomical and functional incorporation into the host brain. These homotypic grafts also induce a wide range of behavioral improvements in experimental animals. In contrast, fetal substantia nigra neurons implanted into the dopamine-depleted neostriatum (heterotypic transplants) generally show a more restricted integration into the host brain and elicit fewer behavioral improvements. Nonetheless, the ability of grafted fetal neurons to survive, differentiate, and partially reconstruct an appropriate and functional neurocircuitry with host systems indicates that there are factors within the adult brain that promote neuronal development and regeneration. Such results have encouraged the clinical use of intracerebral grafts for the treatment of Parkinson's disease. Recent studies have emphasized the use of genetically modified cells and neural cell lines as alternative populations to study and repair the central nervous system. — Fisher, L. J., Gage, F. H. Intracerebral transplantation: basic and clinical applications to the neostriatum. *FASEB J.* 8: 489–496; 1994.

Key Words: graft · brain · excitotoxic lesion · 6-hydroxydopamine · dopamine · genetically modified cells · neural precursors

TISSUES HAVE BEEN IMPLANTED INTO the central nervous system (CNS)[2] for more than a century to assess the function and plasticity of the brain (1). One area of the CNS that has been extensively explored with the grafting technique is the neostriatum, a region of the basal ganglia that is composed of the caudate nucleus and putamen. This structure displays a compartmental organization (2) that is characterized by a heterogeneous distribution of biochemical markers (3). The predominant neuronal cell type present within the neostriatum is the medium spiny neuron (4), so termed because of the dense protuberances present on the dendrites. These cells, which are the principal output neurons of the neostriatum, appear to use γ-aminobutyric acid (GABA) as a neurotransmitter and extend fibers to the substantia nigra and globus pallidus (Fig. 1*A*). The medium spiny cells are, in turn, the primary targets of afferent fibers from the cerebral cortex, thalamus, and substantia nigra. A smaller population of cells within the neostriatum has been identified as cholinergic interneurons and is characterized by large somata, smooth dendrites, and long axonal processes that are extensively ramified.

The neostriatum plays a critical role in motor processing within the brain, as evidenced by the emergence of marked motor dysfunctions when either the neostriatum or striatal afferents are damaged. Such injuries are manifest as the clinical syndromes of Huntington's disease and Parkinson's disease, respectively, and have been mimicked in several animal models. To understand some factors involved in striatal processing, tissues have been implanted into the neostriatum to replace discrete components of the system after experimental damage. In addition to revealing insights into the striatal circuitry, such tissue replacement also provides a method for characterizing factors that contribute to CNS development and the restoration of function after brain damage.

The neural tissues used for grafting typically are obtained from embryonic donors, as cells isolated from the adult brain do not survive well through the grafting procedures. The necessity for using fetal tissues for transplantation raises several interesting issues relating to neural development: *1)* Are signals still present within the adult brain to guide the appropriate differentiation and maturation of grafted fetal neurons? *2)* Can grafted neurons, which may be in a developmentally immature state, recapitulate the neurocircuitry of the adult system? *3)* Can the grafted cells form functionally relevant connections with the host brain? The answer to all of these questions appears to be yes, at least to some extent. However, there are factors that influence the extent to which grafted cells mature and function in a host brain. In particular, cells grafted onto homotypic sites (the area of the brain from which the grafted cells originated) appear to show greater anatomic and functional integration into the CNS than those that are implanted into heterotypic sites (ectopic locations for the grafted cells).

In this review, we discuss studies that have used the grafting technique to explore the development, organization, and plasticity of neural tissues. We focus on two well-characterized model systems of striatal dysfunction: excitotoxic lesions of the neostriatum (5, 6) and 6-hydroxydopamine (6-OHDA) lesions of nigrostriatal afferents (7). Using these model systems, we present some contrasting properties of cells implanted into homotypic and heterotypic sites. Although most of the work in these model systems has centered on the use of fetal neurons for grafting, recent progress with the development and use of alternative tissues in the grafting strategy will also be described. Finally, the application of intracerebral grafts for human therapy, an approach that is already being pursued, is discussed.

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[2]Abbreviations: CNS, central nervous system; GABA, γ-aminobutyric acid; 6-OHDA, 6-hydroxydopamine; TH, tyrosine hydroxylase; NGF, nerve growth factor; EPSP, excitatory postsynaptic potential; IPSP, inhibitory postsynaptic potential.

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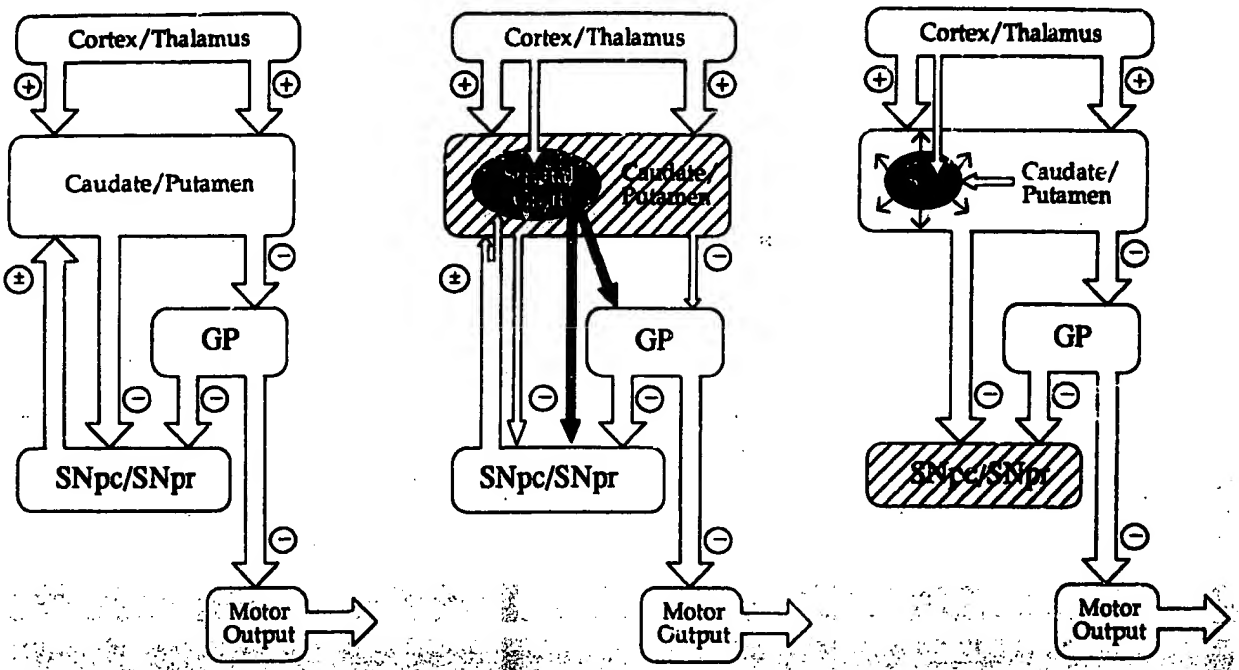

Figure 1. Schematic representations of neostriatal connections in the adult brain. In the intact brain, medium spiny neurons within the neostriatum extend processes to the globus pallidus (striatopallidal neurons) and to the substantia nigra (striatonigral neurons). Inputs to the neostriatum are predominantly excitatory and arise from the cortex and thalamus. The afferent innervation arising from dopaminergic neurons within the substantia nigra pars compacta appears to have both positive and negative influences on neostriatal cells, depending on whether the fibers terminate on striatonigral or striatopallidal neurons, respectively (2) (*A*). Excitotoxic lesions of the neostriatum damage intrinsic neurons and induce the degeneration of striatal efferents to the globus pallidus and substantia nigra. These output pathways are reformed by fetal striatal neurons that are implanted into the damaged area. The homotypic grafts also appear to partially recapitulate corticostriatal and thalamostriatal efferent systems (*B*). Destruction of dopaminergic neurons within the substantia nigra removes dopamine innervation of the neostriatum. Nigral-striatal interactions can be reestablished by implanting fetal nigral neurons ectopically into the neostriatum. These grafted cells reestablish many appropriate interactions with striatal neurons, but are less successful in reconnecting with other regions of the host brain (*C*). Abbreviations: GP, globus pallidus; SNpr, substantia nigra pars reticulata; SNpc, substantia nigra pars compacta; +, excitatory input; −, inhibitory input; ±, mixed excitatory/inhibitory input (figure modified from ref. 64).

HOMOTYPIC NEURAL GRAFTS INTO THE NEOSTRIATUM

Cells within the neostriatum can be selectively destroyed by injecting excitotoxic substances, such as kainic acid or ibotenic acid, into the striatal parenchyma. In rats, such lesions induce the degeneration and death of medium spiny neurons, a shrinkage of the striatum, and a corresponding enlargement of the lateral ventricle (5, 6). Consequently, excitotoxin-treated rats display a range of behavioral deficits, including nocturnal hyperactivity, impaired coordinated use of forelimbs, and cognitive dysfunction.

Striatal neurons that are lost after excitotoxic insult can be effectively replaced by implanting fetal striatal tissue into the damaged regions. Embryonic neural precursors derived from the fetal telencephalon that are implanted into the lesion site show extensive proliferation in the host brain and expand to fill the shrunken region. Within these implants, both medium spiny neurons and large aspiny interneurons have been identified (8–10). In addition, the grafts also typically contain cell types that are unusual for the adult striatum (11). Most of these cells appear to originate from nonstriatal regions of the embryonic brain that are inadvertently retained in the grafting preparation during isolation of the neostriatal primordia (11). This was recently confirmed in a study that explored the morphological properties of graft tissues derived from different regions of the fetal telencephalon (12). Cells dissected from the lateral ganglionic eminence gave rise to grafts that displayed anatomical features that were predominantly striatal whereas grafts containing cells dissected from the medial ganglionic eminence were characterized by a paucity of striatal markers. However, not all of the unusual cells within grafts derived from both of the ganglionic eminences may be nonstriatal, but rather striatal cells that express developmentally immature properties. For example, some cells within the grafts display large somata and labeling for calcium-binding protein (calbindin), which are two properties that are typical of striatal neurons during early postnatal time periods. Further, medium spiny cells within the transplants are often found to display a low density of spines (8, 10), which is characteristic of cells within the perinatal striatum (13). Such observations suggest that neuronal precursors from the fetal striatum differentiate appropriately but may not fully develop when implanted into the adult brain.

A potentially arrested developmental state of grafted cells suggests that extracellular cues play a crucial role in the maturation of neuronal precursors. If the development of

neurons was directed predominantly by intrinsic signals, cells implanted into a novel environment should fully mature regardless of external factors. However, results from a series of electrophysiological studies of the functional properties of fetal striatal cells implanted into the neostriatum indicate that the developmental status of neuronal precursors within transplants may be complicated to define and probably reflects a combination of both internal and external influences. For this work, embryonic striatal tissue was implanted into the kainic acid-treated neostriatum of adult rats. Using in vivo recording techniques, medium spiny neurons identified within the grafts by intracellular injection of the marker biocytin were found to display a variety of membrane properties that are unusual for mature spiny neurons (14; Table 1). Specifically, input resistances of the cells were two- to fivefold higher than normal and the time constants were unusually long. These properties appeared to reflect an absence of the anomalous rectification prominent in spiny neurons in the adult neostriatum (15). Because anomalous rectification is a property that appears late in the development of spiny cells (13, 16), such results suggest either that the intrinsic developmental time course of grafted neurons has been altered (delayed) or that the cells may not receive adequate guidance from the extracellular environment. Of these two possibilities, the latter seems more likely for several reasons. First, the grafted cells never appear to develop anomalous rectification even at postgraft intervals of as long as 6 months (14). Second, other physiological properties of the grafted spiny cells appear to develop with a time course that is similar to that displayed by striatal neurons in situ. Specifically, it has been shown that grafted striatal neurons express a slowly inactivating potassium conductance that is developmentally regulated and appears only beyond 4 weeks postnatal (17). The presence of mature potassium currents in the same population of grafted neurons that show immature (no) anomalous rectification offers some insights into striatal neurons and grafted cells in general. At one level, such observations highlight the utility of the grafting technique for identifying properties of neurons whose development may be acutely dependent on appropriate interactions with environmental signals: anomalous rectification may be expressed by neostriatal neurons only in response to specific external cues, whereas mature potassium currents can develop either independently of extracellular signals or with little external direction. At a more fundamental level, divergent properties in the same cells suggest that the developmental status of grafted neurons may be elusive. The cells may be displaying a unique developmental state that is characterized by a range of features that span a broad developmental time frame. Alternatively, the mixture of developmental properties expressed by the grafted neurons may simply reflect the extent to which the cells incorporate into the host circuitry. Indeed, both the host innervation of the grafts and the response of the implanted cells to host stimuli are subnormal (see below). Distinguishing between these possibilities remains an area of continuing investigation.

Although grafted striatal neurons express some properties that differ from mature counterparts, they show a remarkable integration into the striatal circuitry (Fig. 1*B*). Implanted neurons that appear to express a GABAergic phenotype extend fibers both to the globus pallidus and to the substantia nigra (18, 19). In turn, cells within the grafts are innervated by all of the major striatal efferent pathways originating from the cortex, thalamus, and substantia nigra (18, 20–22). However, this host innervation of the graft is typically sparse and the ingrowing fibers tend to form an unusually high incidence of synaptic connections onto dendritic shafts (21, 22). Such atypical innervation patterns appear to be reflected in the electrophysiological responses of the grafted cells to host stimuli. Specifically, stimulation of the cortex and thalamus has been found to evoke an unusual, pronounced, and transient inhibitory postsynaptic potential (IPSP) in the grafted neurons that gradually disappears and is replaced by an excitatory postsynaptic potential (EPSP), which is smaller than normal and lacks the after-hyperpolarization that characterizes spiny neurons in situ (14). Similar responses have been described for neostriatal neurons during the first few postnatal weeks (13; see **Fig. 2**, Table 1), suggesting that the afferent innervation of the grafts is developmentally immature. Nonetheless, the partial recapitulation of neostriatal circuitry that occurs postgrafting confirms that the adult brain is capable of neuronal regeneration or sprouting and further suggests that fetal tissue grafts provide potent tropic (axon guiding) and/or trophic (nutrient) factors that are involved in this anatomical and functional plasticity.

TABLE 1. *Characteristics of adult, neonate, and grafted neurons within striatal tissues*[a]

Property	Adult	Neonate	Graft
Anomalous rectification	Yes	No	No
After-hyperpolarization	Yes	No	No
Transient IPSP	No	Yes	Yes
EPSP latency	Short	Long	Long
I_{AS} potassium current	Yes	No	Yes
Spine density	High	Low	Low-moderate

[a]Table modified from ref 13.

Despite some of the unusual anatomical and electrophysiological characteristics of homotypic striatal grafts, the functional effect of these implants on the host system is profound. The hyperactivity displayed by the kainic acid-treated rats after transplantation is reversed, there are improvements in skilled motor coordination, and some cognitive abilities return toward normal levels (23–26). There are always some restrictions in the extent to which behavioral abnormalities are recovered, however, which has been suggested to reflect the incomplete circuitry established between graft and host tissues. This notion has recently been drawn into question by an important study that revealed that rats must learn to use a transplant in order to benefit from the graft-host connections (27). In these experiments, rats treated with kainic acid that either were not grafted or received implants of homotypic fetal tissue were tested for their ability to perform a visual response task that is impaired in rats with striatal lesions (28). The grafted rats were initially quite impaired on this task when assessed 6 months post-transplantation, but showed a rapid amelioration of this deficit with repetitive training. These improvements did not appear to reflect the formation of additional graft-host circuitries, as the enhancements occurred over a matter of a few days. Rather, these results suggest that information learned by the host brain must be conveyed to the grafted neurons to fully recruit their participation in CNS functioning.

HETEROTYPIC NEURAL GRAFTS INTO THE NEOSTRIATUM

The substantia nigra pars compacta sends a large dopaminergic projection to the neostriatum. This pathway

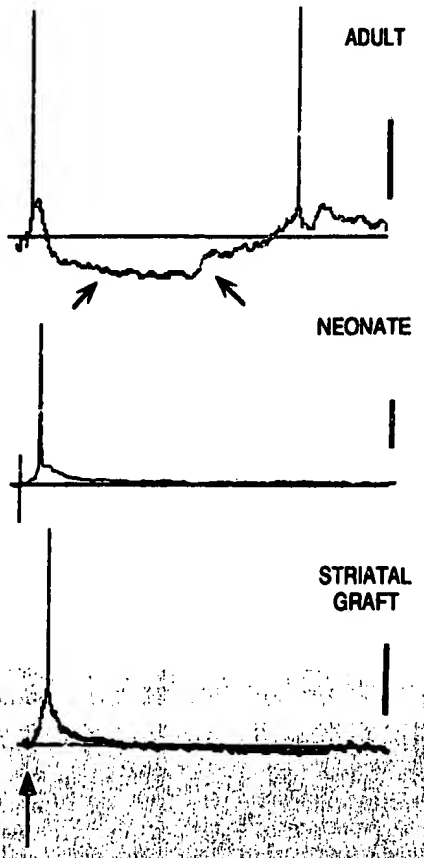

Figure 2. Representative neuronal responses within the adult, neonate, and grafted neostriatum of rats after cortical stimulation. Medium spiny cells within the adult neostriatum display a short latency EPSP, followed by a long-lasting hyperpolarization (indicated by arrows). This after-hyperpolarization typically is not elicited by cortical stimulation within the developing neostriatum until rats are beyond 30 days postnatal. As in neonates, the evoked after-hyperpolarization is absent in spiny neurons within homotypic neostriatal grafts. Calibration bars = 20 mV. (Traces from adult neostriatum and striatal graft were modified from ref 14. Trace from neonate courtesy of J. M. Tepper and F. Trent.)

can be selectively eliminated by injecting the catecholamine neurotoxin 6-OHDA into the nigrostriatal track (7). One of the characteristic behavioral consequences of unilateral dopamine loss from the neostriatum is the tendency of the treated rats to walk in a circle when injected with dopamine agonists. This locomotor pattern has been termed rotational behavior and provides an index of dopamine levels within the denervated neostriatum (29). This behavior has thus frequently been used to assess the extent to which intrastriatal grafts of fetal substantia nigra tissue can restore dopamine content and function in the neostriatum.

Fetal substantia nigra neurons grafted ectopically in or adjacent to the dopamine-denervated neostriatum survive well and integrate anatomically into the host brain (Fig. 1*C*). However, some of these interactions are atypical of nigral connections in the adult brain. When transplants are assessed at long postgraft intervals that should reflect a stabilized host-graft circuitry, the grafts generally show sparse afferent input from CNS structures that normally innervate the substantia nigra in situ. There is some cortical input to neurons deep within the grafts but striatal fibers remain predominantly confined to regions of the graft-host interface (30). The grafted dopamine neurons extend axons as far as 2 mm into the host neostriatum, where many form normal-looking contacts onto medium spiny neurons. However, there is also a disproportionately high number of synapses formed onto the dendritic shafts of these cells (31, 32). Furthermore, processes from the grafted cells have also been found to terminate in a completely abnormal manner on the somata of the aspiny interneurons (31).

Unusual anatomical connections between grafted nigral cells and the host brain may reflect a developmentally immature status of the implanted neurons and/or new neural circuitry. Indeed, there is evidence to suggest that dopamine neurons within the transplants may not fully mature within the adult host. As long as 7 months post-transplantation, these cells have been found to be covered with bulbous spines and glial processes in a manner characteristic of neonatal dopamine neurons (33). Also, the spontaneous electrophysiological activity of grafted nigral neurons is marked by extremely slow-firing, wide-action potential waveforms and doublet bursting patterns through 5 months postgrafting (34), a distinctive set of properties that has been found in substantia nigra neurons only during the first few weeks after birth (35). In contrast, the metabolism of dopamine within the grafted cells is characteristic of intrinsic nigrostriatal neurons (29), and the implanted neurons respond to a challenge with dopamine agonists in a normal manner (36, 37). Such findings are consistent with observations from homotypic striatal grafts that suggest that some properties of neuronal precursors are intrinsically determined, whereas others may fully develop only in response to appropriate extracellular cues. That electrophysiological properties of dopamine neurons are partially determined by afferent connectivity is suggested from observations that grafted nigral cells display more mature electrophysiological properties at long (>5 months) postgraft intervals (38). The particular influence of extrinsic signals on early neuronal cells has been further suggested from studies comparing the growth patterns of fetal nigral neurons in different striatal environments. Fiber outgrowth from cells implanted into the 6-OHDA-treated neostriatum is greater than that seen from nigral neurons grafted onto the intact neostriatum but less than the extensive innervation patterns that typify cells grafted onto the neostriatum of neonates (39, 40). As the fetal neurons were subjected to the same experimental manipulations before grafting in these studies, the diverse growth patterns displayed by the cells under different environmental conditions highlight the importance of extracellular factors on neuronal development and/or plasticity.

Fetal nigral neurons implanted into the 6-OHDA-denervated neostriatum ameliorate only a subset of the behavioral abnormalities that emerge after the lesion. The first described effect of substantia nigra grafts on dopamine-depleted rats, which has been replicated many times, was the reduction or complete abolition of drug-induced rotational behavior (1). Improvements were found to be dependent both on the position of the grafts in the neostriatum and on the presence of dopamine neurons within the grafts. Another impairment that recovers after grafting is sensory responsiveness to peripheral stimuli. This improvement has also been linked to a precise neostriatal locus and reaffirms the usefulness of grafts in identifying functional domains within the brain (41). Among those behaviors that typically remain impaired after transplantation are the coordinated use of forelimbs (42) and an activity termed disengage behavior (43). The persistence of these deficits postgrafting has been

suggested to reflect the limited extent to which the implanted neurons anatomically and functionally integrate int the host circuitry. If this hypothesis is correct, then further recoveries should be realized if graft-host integration could be improved. This is precisely the finding of two recent studies that described a technique for achieving widespread distribution of grafted neurons and their associated processes throughout the neostriatum (44, 45). In these experiments, fetal nigral neurons suspended in grafting solution were injected into the neostriatum either by using standard transplantation procedures (a few deposits in one or two sites) or in 18 small deposits. The dispersed micrografts displayed greater reinnervation of the host neostriatum and increased dopamine levels above that observed with the macrografts. Most important, however, was the finding that skilled forelimb use and disengage behavior were both improved by the small, multiple grafts. These results indicate that, under appropriate conditions, fetal neurons have the capacity for extensive incorporation into the adult CNS and for recapitulating neural circuitries in the damaged brain that have profound functional consequences for an implanted host.

NON-NEURAL TRANSPLANTS INTO THE NEOSTRIATUM

Early work with fetal nigral neurons implanted into 6-OHDA-treated rats suggested that simply increasing the amount of dopamine within the damaged neostriatum may be sufficient to ameliorate some behavioral abnormalities of the experimental animals. Thus, many studies have explored the potential for using other catecholamine-producing cells as a donor material for grafting. The primary focus of this approach is the adrenal gland, which contains cells that increase dopamine synthesis when removed from the influence of corticosteroids. Although non-neural in origin, these cells survive moderately well within the brain and show evidence of dopamine production after grafting (46). The cells do not generally extend processes into the host parenchyma, which limits th r sphere of influence in the host brain. However, even suc local delivery of dopamine is able to induce substantial reductions in the abnormal rotational behavior of 6-OHDA rats. These results suggested that passive dopamine replacement within the neostriatum is sufficient to restore simple motor processing within the damaged brain.

This conclusion has recently been questioned with results suggesting that recovery of some rotational behaviors cannot be directly linked to dopamine replacement within the brain. Rats with unilateral 6-OHDA lesions exhibit circular turning either in ipsiversive (toward the lesion side) or contraversive (toward the intact side) directions, depending on the drug administered to the animals. Specifically, rats turn in the direction opposite to the neostriatum that is most active. Thus, the dopamine-releasing drug amphetamine increases dopamine levels within the intact neostriatum and induces ipsiversive rotations, whereas the dopamine receptor agonist apom rphine predominantly stimulates postsynaptic dopamine receptors that increase in the denervated neostriatum after the lesion and induces contraversive rotations (47, 48). When 6-OHDA-treated rats are implanted with adrenal cells and tested for rotational behavior, both apomorphine- and amph tamine-induced turning are reduced. However, only the decreased rotations that are bserved in response to amphetamin have been co.elated with increased dopamine levels within the neostriatum (49). Reductions of apomorphine-induced turning occurs in th absence of increased levels f dopamine ither in serum (50) or within the neostriatum (49). Such results have been suggested t indicate that decreases in apom rphine turning reflect the influence of nond paminergic substances, such as trophic factors, that are released from grafted cells and recruit the involvement of the host brain. An alternative explanation, however, is that reductions in apomorphine turning may occur when dopamine content within the neostriatum is supplemented by only very small quantities. A corollary of this possibility is that impro ements in apomorphine rotational behavior may be acutely dependent on the location of the grafted cells within the neostriatum.

Evidence that supports the possibility that recovery of apomorphine rotations may occur with low-level and/or site-specific supplementation of dopamine within the neostriatum has been provided by work with genetically modified cells implanted into the brain. The powerful advantage of engineered cells over other cell types for grafting is that a population of cells can be genetically modified to express only one single factor hat is different from the original parent population. Thus, changes in function or behavior that occur after the intracerebral implantation of the modified population, but not its parent population, can be linked to the presence of the single engineered product.

GENETICALLY MODIFIED CELLS

A series of studies has focused on genetically modifying cells to express the catecholamine synthetic enzyme tyrosine hydroxylase (TH). The majority of cells that have been modified to express TH produce the dopamine precursor l-dopa, as the cells typically lack the enzyme (L-aromatic amino acid decarboxylase) necessary for dopamine synthesis. The absence of this second enzyme is not critical, as L-dopa released from the TH-expressing cells after grafting appears to be rapidly converted to dopamine in the host neostriatum (51). Regardless of the cell type used for genetic modification, TH-expressing cells have routinely been found to induce reductions in apomorphine-induced turning when compared with nonmodified parent populati ns (52). Further, such recovery has been linke to a specific regional placement of the grafts within the neostriatum (53). These results provide strong evidence that dopamine supplementation within precise areas of the neostriatum is associated with amelioration of the contraversive turning elicited with apomorphine. However, these findings do not eliminate the possibility that host dopaminergic fibers within the neostriatum that may be spared after 6-OHDA lesion could also play a role in this re very.

In addition to providing some insight into the role of dopamine in neostriatal functioning, genetically modified cells implanted into the neostriatum have been used to reveal factors that are involved in neural regeneration in the adult brain. For example, skin fibroblasts obtained from rats have been genetically modified to produce the neurotrophic molecule nerve growth factor (NGF) and then implanted into the intact neostriatum of adult rat hosts (54). Fibroblasts that were not genetically manipulated were grafted for control cor parisons. Both populations of peripherally derived cells survived equally well within the CNS environment, but showed markedly different effects on the host brain. Specifically, the NGF-producing grafts were characterized by a dense ingrowth of fibers that showed immunohistochemical labeling for NGF receptor and appeared t be cholinergic. In contrast, nonmodified fibroblast grafts were devoid f such processes. This observati n was consistent with previous work indicating that, in addition t its well-known effect

on ch linergic survival, NGF acts as a tropic m lecule f r adult ch linergic ax ns. M st striking, however, was the finding that host axons appeared to use astrocytic processes that had penetrated into the NGF grafts as a preferred substrate f r growth (Fig. 3). Notably, the lack of axonal ingrowth on astrocytic processes within the control grafts indicated that astr cytes may act as a conducive substrate for axonal regeneration only in the presence of appropriate exogenous factors. Such findings refute the notion that glial scars are necessarily a barrier to axonal regeneration, and they suggest methods for exploiting the growth-permissive properties of reactive astrocytes after injury.

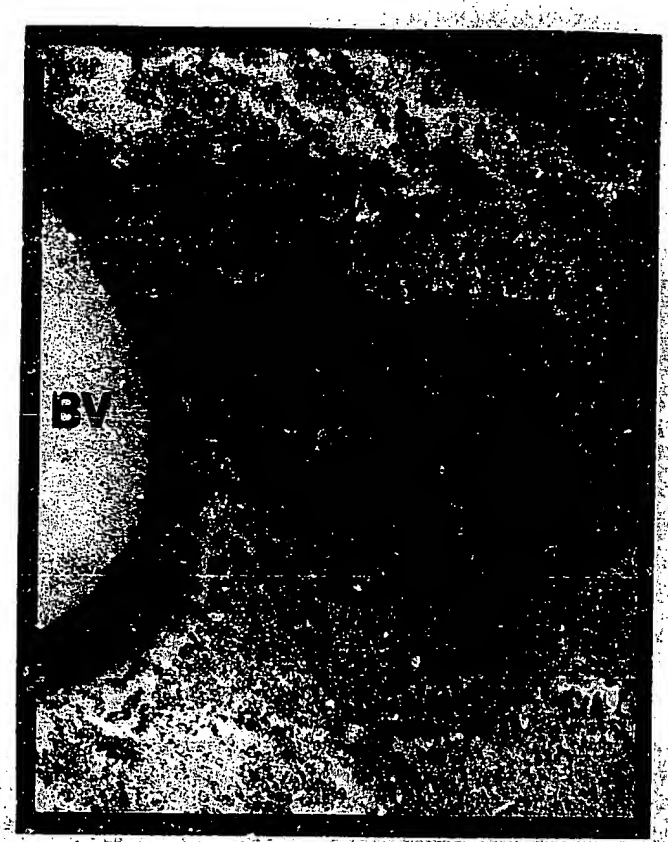

Figure 3. Electon micrograph of neuronal processes within grafts of NGF-producing fibroblasts. Host-derived axons (some marked with asterisks) that grow into transplants containing NGF-producing cells are always associated with astrocytes (AS) rathc than other conducive substrates in the graft milieu such as collagen (C) or basal lamina of blood vessels (BV) (figure modified from ref 54).

CLINICAL WORK

Parkinson's disease is characterized by the degeneration of dopaminergic neurons within the substantia nigra and the subsequent loss of dopamine content within the neostriatum. Currently, the most common method for treating the disease is the oral administration of the dopamine precursor L-dopa, which is used instead of dopamine because it can cross the blood-brain barrier before it is metabolized. Although this compound can control many adverse symptoms of the disease, long-term treatment typically results in diminished and/or fluctuating responsiveness to L-dopa and the emergence of additional motor dysfunctions. Results indicating that intrastriatal grafts of dopaminergic tissues ameliorate s me of the behavioral abnormalities of 6-OHDA rats have suggested a novel approach for treating the dopamine depletion that occurs within the neostriatum of patients with Parkinson's disease. Work with adrenal cells in particular indicated that an autologous source of catecholamine-rich tissue could be obtained from the patients themselves to minimize potential problems with immunological responses. Although issues concerning the survival and function of adrenal grafts within rats were unresolved, clinical grafting of adrenal tissue was initiated on Parkinsonian patients almost a decade ago (46). Subsequently, there have been hundreds of clinical transplants of adrenal tissue, with most patients receiving little ben from the grafts. Moreover, the significant morbidity and mortality associated with this transplantation procedure indicate that autologous adrenal grafts currently are neither safe nor effective as therapy for Parkinson's disease.

The minimal clinical effects of adrenal grafts implanted into patients with Parkinson's disease were consistent with the limited effectiveness of these tissues in rats with experimental dopamine depletion. A seemingly more appropriate candidate tissue for a transplantation therapy in Parkinson's disease is fetal nigral neurons. As described above, these cells can induce a broad range of improvements in sensory-motor functioning within the CNS, particularly when the new methods for implantation are used. A disadvantage of this tissue for human therapy is the necessity for using aborted material. In addition to generating ethical debates (55), the use of nonautologous tissue poses the risk of host rejection of the foreign cells after transplantation. However, the lack of effective long-term therapy for Parkinson's disease has encouraged the investigation of neural transplants as an alternative approach for treating this disrder. T date, more than 100 Parkinsonian patients have received implants f neural tissue from embryonic substantia nigra (56). Marked improvements, as defined by increased responsiveness to L-dopa therapy (lower doses required f r controlling sympt ms and less fluctuations), have been observed in a significant number of cases. Recently, impressive results have also been reported for two Parkinsonian-like patients that suffered a substantial loss of dopamine neurons within the brain after self-injection of a potent catecholamine neurotoxin (57). Although neuronal grafting thus appears to offer a promising therapeutic strategy for Parkinson's disease, questions remain concerning the technical aspects of the transplantation, including the need for immunosuppression, the amount of tissue required, and the appropriate place to implant the fetal tissue within the neostriatum. These issues continue to be addressed in animal models to help clarify those parameters that increase the survival and functioning of human fetal nigra neurons implanted in the adult brain.

In addition to Parkinson's disease, the extent to which fetal striatal cells have been able to structurally and functionally reconstruct the damaged neostriatum has clear implications for developing a therapy for humans with Huntington's disease. However, several aspects of this disease make it challenging to treat. First, it is difficult to identify a point at which grafts should be used to intervene in the striatal neurodegeneration. Second, as the mechanisms responsible f r killing striatal neurons remain unknown, it is possible that newly implanted striatal neurons may succumb to the same disease pr cess. However, even if destruction of the grafts does occur, the implanted cells may provide s me degree of striatal integrity that results in l nger protection to the compromised system. Thus, interest in using grafts for Huntington's disease remains high and such a strategy will probably be developed with caution as more is learned from work with Parkinsonian patients that have received fetal transplants.

FUTURE DIRECTIONS

One of the newest developments in the grafting technique has been the introduction of multipotential neural precursors as an alternative donor material for transplantation (58, 59). These cells were generated by inserting an oncogene into proliferating cells isolated from the embryonic or neonatal brain. The cells could then be easily grown and manipulated in vitro and well characterized before implantation. When grafted onto the CNS, such cells have demonstrated a remarkable plasticity by differentiating into diverse neuronal and glial cell types that are appropriate for the site of injection. In addition to providing crucial insights into the factors involved in neural development, the generation of immortalized precursor populations that are capable of differentiating into multiple CNS cell types in vivo has significant implications for the treatment of neural dysfunction. Such cells may be manipulated toward a lineage that synthesizes factors of interest and may be used in grafting strategies to replace substances that are lost after injury or in neurodegenerative disease. Alternatively, precursor cells may be directed to a particular neuronal lineage and used to functionally recapitulate damaged neural systems. Finally, genetic modification of precursor populations may provide a method for introducing therapeutic gene products into the CNS.

Although immortalization techniques have proved useful for generating large quantities of precursor cells for study and transplantation, the genetic modification of cells may alter vital cellular properties. Thus, recent reports that epidermal growth factor and basic fibroblast growth factor induce the proliferation of nonimmortalized neural populations in vitro from the embryonic (60, 61) and adult brain (62, 63) provide an important strategy for expanding and maintaining primary neural populations for prolonged periods in culture. Such results have particular implications for human therapy, as trophic factors may potentially be used to generate perpetual lines of multipotential cells from the human CNS that may serve as a universal donor material for a range of neurodegenerative disorders. At a more fundamental level, work with nonimmortalized neural precursors will provide additional insight into the properties of CNS stem cells and increase the range of neural populations that may be used as a tool for exploring the development, function, and plasticity of the CNS. FJ

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